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**6. AUTHOR(S)**
Dr G. S. Sayler

**7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)**
Center for Environmental Biotechnology
The University of Tennessee
10515 Research Drive, Suite 100
Knoxville, Tennessee 37932-2567

**8. PERFORMING ORGANIZATION REPORT NUMBER**

**9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)**
AFOSR/NL
110 Duncan Avenue, Suite B115
Bolling AFB DC 20332-0001
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The goal of the research supported by this grant is to determine the role that biosurfactants and biosurfactant-producing microorganisms may play in enhancing the rate and/or extent of polycyclic ıromatic hydrocarbon (PAH) biodegradation in particulate media. Biosurfactants, which are ...urface-active compounds produced by certain bacterial strains, have been shown to increase the ...pparent aqueous solubility of sparingly-soluble organic contaminants including PAHs. The ...iodegradation rate is often controlled by the aqueous concentration which in turn may be ...ontrolled by sorption/desorption equilibrium in particulate matrices. Biosurfactants may function ... another phase for the contaminant to partition into, thereby increasing the available pool of the ...ntaminant and, thus, its rate of degradation.



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Contract Number F49620-92-J-0333
First Annual Technical Report (June 1, 1992 - May 31, 1993)
(FY91 AASERT) Molecular Probes and Bioluminescent Reporters in
Ecological Optimization of Biodegradation

G.S. Sayler
Center for Environmental Biotechnology
University of Tennessee, Knoxville

The goal of the research supported by this grant is to determine the role that biosurfactants and biosurfactant-producing microorganisms may play in enhancing the rate and/or extent of polycyclic aromatic hydrocarbon (PAH) biodegradation in particulate media. Biosurfactants, which are surface-active compounds produced by certain bacterial strains, have been shown to increase the apparent aqueous solubility of sparingly-soluble organic contaminants including PAHs. The biodegradation rate is often controlled by the aqueous concentration which in turn may be controlled by sorption/desorption equilibrium in particulate matrices. Biosurfactants may function as another phase for the contaminant to partition into, thereby increasing the available pool of the contaminant and, thus, its rate of degradation.

To achieve the proposed goal, bacterial strains containing specific degradative genes and bioluminescent reporter systems will be used to monitor the effectiveness of biosurfactant-producing strains for biodegradation of aromatic hydrocarbon contaminants in environmental simulations. These genetic marker systems which allow for the quantitation of degradative gene frequency and activity will be used to address specific issues such as the effect of the ratio of the degrader population to the non-degrader population and the effect of various environmental conditions including nutrient status and pH on the PAH biodegradation process.

To date, work has focused on strain selection and modification, with two major directions being taken. The first has involved constructing a strain with the combined abilities to produce biosurfactant and to degrade various PAHs including naphthalene, phenanthrene and anthracene. Three strains are on hand which are known to produce biosurfactant on glycerol, *n*-alkanes or crude oil substrates. One strain, *Pseudomonas aeruginosa* 652 produces a rhamnolipid surfactant which increased the solubility of phenanthrene and resulted in increased desorption of phenanthrene from organic-coated clay particles relative to control treatments. Since relatively less is known about the other two biosurfactant-producers, one of these strains is being characterized taxonomically and genetically, in addition to isolation and characterization of its produced biosurfactant. Future work will involve incorporation of a plasmid which encodes genes for PAH degradation as well as the *lux* gene cassette for bioluminescence monitoring into two of these strains.

The second direction has involved isolating a strain with the combined abilities to produce biosurfactant and to degrade PAHs. An initial screening for biosurfactant activity was performed on strains which were previously isolated for their ability to degrade various PAHs. One strain which can mineralize naphthalene, phenanthrene and anthracene also showed promise for excreting a surface-active compound which increased the desorption of phenanthrene from organic-coated clay surfaces. As part of this approach, strains have been isolated from creosote enrichments in which growth is observed and the creosote is emulsified. Since creosote is immiscible with water and is largely composed of PAHs, these isolates which grow on creosote and cause its emulsification would be expected to produce some sort of surface-active compound to achieve this, as well as to directly metabolize a portion of the PAH components. The strain selected from this procedure will also be modified to include the *lux* genes for bioluminescence monitoring in complex environmental matrices.

The strain which has been selected and marked genetically along with strains mutated in biosurfactant production and PAH degradation will be used in batch and continuous-flow (column) systems composed of subsurface soil or aquifer material contaminated with PAH mixtures to evaluate the effect of biosurfactants on PAH desorption and biodegradation. Corollary studies will be performed with synthetic surfactants to determine whether biologically-produced or synthetic surfactants are more effective in enhancing PAH bioavailability and biodegradation.

Academic Progress

The student supported on Contract No. F49620-92-J-0333 has made satisfactory academic progress towards her Ph.D. requirements. The following courses were taken with the appropriate letter grades indicated below:

Biochemistry 511-Advanced Concepts in Protein Structure, Protein Function and Intermediary Metabolism (A)
Biochemistry 512-Advanced Molecular Biology (A)
Microbiology 470-Microbial Ecology (A)
Microbiology 670-Advanced Topics in Environmental Microbiology (A)
Mathematics 405-Models in Biology (A)
Chemistry 431-Radioactive Tracer Techniques (A)